Phytopathogenic Mollicutes
Vol. 9 (1), June 2019, 19-20

doi: 10.5958/2249-4677.2019.00010.0

IndianJournals.com
A product of Diva Enterprises Pvt. Ltd.

Worldwide

Unveiling subgroups of 16SrIII phytoplasmas affecting *Trillium* sp. and *Melothria pendula* in Brazil and Canada

Helena Guglielmi Montano[1] and Yaima Arocha Rosete[2]

[1]Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro, Brazil
[2]Sporometrics Inc, Toronto, Canada

Abstract

Melothria pendula and *Trillium* spp. are important weed species in Brazil and Canada. *M. pendula* plants showing symptoms of chlorosis, little leaf and leaf malformation and *T. grandiflorum* and *T. erectum* exhibiting virescence were tested for phytoplasma presence by nested PCR with primers that amplify the 16S rRNA, ribosomal protein, and *secA* genes, and subjected to RFLP and sequencing analyses. A phytoplasma of group 16SrIII was identified from *M. pendula* and classified as a new variant of subgroup 16SrIII-U. The 16SrIII phytoplasmas, first reported in *T. grandiflorum* and *T. erectum* in Ontario in 2016 were classified as members of the subgroup 16SrIII–F. Besides, one strain from each *Trillium* species examined, corresponded to a new variant of subgroup 16SrIII-F (for *T. grandiflorum*) and a new variant of subgroup 16SrIII-J (for *T. erectum*). Results refer to the first report of a phytoplasma affecting *M. pendula*, and the identification of new variants of 16SrIII phytoplasma subgroups in *Melothria* and *Trillium* species, which may pose a threat for the spread of 16SrIII phytoplasmas to other plant species grown nearby.

Keywords: *Melothria*, *Trillium*, 16SrIII phytoplasma, subgroup variants

Introduction

The X-disease group (16SrIII) of phytoplasmas is one of the most economically important and widely spread groups across Europe, East Africa, and the Americas. They affect many different plants, particularly *Prunus* species and differ greatly in their geographical origin, plant host/insect vector relationships, and symptoms (Saccardo *et al*., 2012). In Canada, 16SrIII phytoplasmas were reported in 1941 in the Ontario province associated with peach X-disease, while there is a vast list of plant species in Brazil known to host this phytoplasma group, which include cassava (Oliveira *et al*., 2014), *Melia azedarach* (Duarte *et al*., 2009), citrus (Wulff *et al*., 2019) and others. *Trillium* species (family Melanthiaceae) are commonly used as medicinal plants, and particularly *T. grandiflorum* serves as the emblem and official flower of the Ontario. In Brazil, *Melothria pendula* (Cucurbitaceae family), known as "pepininho" is a weed with laxative properties and grows wild in household gardens and backyards. This study presents the results on the characterization of phytoplasmas of group 16SrIII found in *Trillium* sp. in Canada, and *M. pendula* in Brazil.

Materials and Methods

Surveys for phytoplasma presence were conducted in private gardens of the Seropédica area in Rio de Janeiro, Brazil, during 2016. Leaves, petioles and flowers were collected from *M. pendula* plants showing symptoms of chlorosis, little leaf and leaf malformation (Figure 1A). In Canada, *Trillium* species (*T. grandiflorum* and *T. erectum*) exhibiting typical virescence symptoms (Figures 1C and 1D), collected from a private property in Ontario in 2016 were subjected to multigene characterization. Total DNA extracted by the FAST DNA Spin Kit (MP Biomedicals, USA) was tested by nested PCR (Illustra Ready-to-go-PCR, GE Healthcare, UK) with universal phytoplasma 16S rDNA primers R16mF2/R1 and R16F2n/R2 (Gundersen and Lee, 1996). The phytoplasma secretion protein gene, *secA* was amplified with primers SecAfor1/SecArev3 nested with SecAfor5/SecArev2 (Dickinson and Hodgetts, 2013), and the ribosomal protein genes were amplified with rp(III)F1/R1 primers (Martini and Lee, 2013). PCR amplicons were purified (Omega Bio-Tek, USA) and cloned (pGEM-T Easy Vector, Promega). Plasmid DNA was extracted, purified (GenElute Plasmid Miniprep Kit, Sigma-Aldrich, USA) and sequenced with primers M13F/M13R (Centre for the Analysis of Genome Evolution and Function, University of Toronto, Canada). The consensus sequences were compared to GenBank phytoplasma sequences (https://blast.ncbi.nlm.nih.gov) and used in phylogenetic analysis with MEGA version 7.0. The

Corresponding author e-mail: Yaima Arocha Rosete (yarosete@sporometrics.com)

R16F2n/R2 sequences were subjected to *i*PhyClassifier (https://plantpathology.ba.ars.usda.gov) for phytoplasma group and subgroup affiliation while *secA* and *rp* gene sequences were analysed with pDRAW32 (http://www.acaclone.com). Ten microliters of PCR amplicons were digested with appropriate restriction endonucleases (New England Biolabs, Canada). RFLP profiles were visualized in 3% agarose gels stained with SYBR Safe (Invitrogen, USA) in an UV gel documenter (Alpha Innotech, USA).

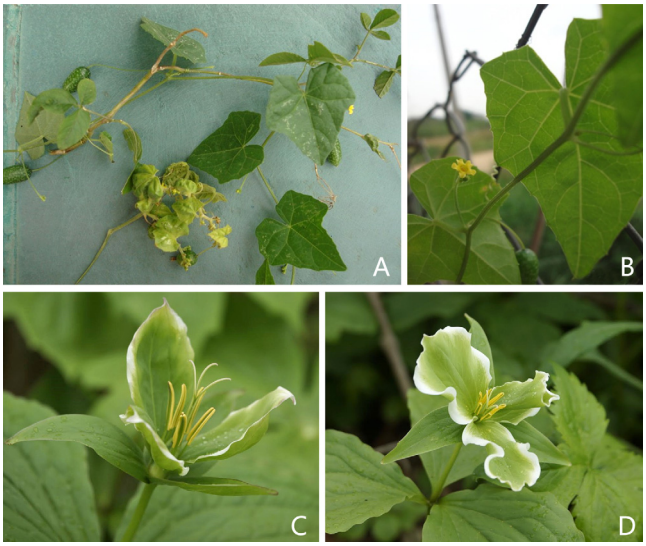

Figure 1. Chlorosis, little leaf and leaf malformation (A) in *M. pendula* versus symptomless plants (B). Virescence in *T. erectum* (C) and *T. grandiflorum* (D) flowers.

Results

The 16S rDNA sequences from the four symptomatic *M. pendula* plants were identical. The consensus sequence deposited in GenBank (accession number MK108032) of the *M. pendula* phytoplasma shared a 99% identity with those of the 16SrIII phytoplasma group (X-disease), and was placed within the 16SrIII cluster as a '*Candidatus* Phytoplasma pruni'-related strain. The *i*PhyClassifier analysis indicated that the virtual R16F2n/R2 RFLP pattern of the *M. pendula* phytoplasma was most similar to that of the group 16SrIII, subgroup U (GenBank accession number HM589213), with a similarity coefficient of 0.98, which suggested that this strain is a variant of the phytoplasmas in subgroup 16SrIII-U. The *i*PhyClassifier analysis from the *T. grandiflorum* (GenBank accession number KX470428) and *T. erectum* (GenBank accession numbers KX470427, KX470429 and KX470430) ribosomal sequences supported their 16SrIII-F subgrouping as '*Ca.* P. pruni'-related strains with a 99% sequence identity. Based on 0.98 RFLP similarity coefficients, the phytoplasma from *T. grandiflorum* was suggested as a new variant of the 16SrIII-F subgroup, and that from *T. erectum*, as a new variant of the 16SrIII-J subgroup. Results from *i*PhyClassifier were confirmed by the analysis of *secA* and *rp* gene sequences and phylogeny.

Discussion

Weeds are proven to be one of the best means for phytoplasma survival, particularly in crop systems. Therefore, knowing those secondary/alternative plant hosts is critical to support an effective management and control of phytoplasma diseases, and to help mitigating the impact of chemical use to crops reducing the environmental impacts. *M. pendula* was confirmed as a new host for phytoplasmas worldwide and was also added to the Brazilian inventory of alternative phytoplasma host species (Montano and Arocha Rosete, 2019). Results suggest that there may be epidemiological factors associated with the presence of mixed infection between the subgroups 16SrIII-F and 16SrIII-J in *T. erectum*, as well as with the occurrence of new variants related to the two subgroups in both *T. grandiflorum* and *T. erectum*. The presence of '*Ca.* P. pruni'-related subgroups and their variants in *M. pendula* in Brazil and *Trillium* species in Canada poses a threat for other weed species grown in nearby private gardens in Rio de Janeiro and in the province of Ontario, which may contribute to the spread of diseases associated with 16SrIII phytoplasmas.

Acknowledgements

This work was supported by the funds from the Inter-American Institute for Cooperation on Agriculture (IICA-Canada): "2018 Research & Internship Assistance Program for Agriculture in the Americas" granted to Dr. H. Guglielmi Montano, UFRRJ, Brazil, and conducted at Sporometrics Inc., Toronto, Canada.

References

Dickinson M and Hodgetts J. 2013. PCR analysis of phytoplasmas based on the *secA* gene. *Methods in Molecular Biology*, 938: 205-215.

Duarte V, Silva EG, Hass ICR, Bedendo IP and Kitajima EW 2009. First report of a group 16SrIII-B phytoplasma associated with decline of china tree in Brazil. *Plant Disease*, 93(6): 666.

Gundersen DE and Lee I-M 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer sets. *Phytopathologia Mediterranea*, 35: 144-151.

Martini M and Lee I-M 2013. PCR and RFLP analyses based on the ribosomal protein operon. *Methods in Molecular Biology*, 938: 173-188.

Montano HG and Arocha Rosete Y 2019. First report of the identification of a '*Candidatus* Phytoplasma pruni'-related strain of phytoplasma in *Melothria pendula*. *New Disease Reports*, 39: 5.

Oliveira SAS, Abreu EFM, Araújo TS, Oliveira EJ, Andrade EC, Garcia JMP and Álvarez E 2014. First report of a 16SrIII-L phytoplasma associated with frogskin disease in cassava (*Manihot esculenta* Crantz) in Brazil. *Plant Disease*, 98: 153.

Saccardo F, Martini M, Palmano S, Ermacora P, Scortichini M, Loi M and Firrao G. 2012. Genome drafts of four phytoplasma strains of the ribosomal group 16SrIII. *Microbiology*, 158: 2805–281.

Wulff NA, Fassini CG, Marques VV, Martins EC, Coletti DAB, Teixeira DDC, Sanches MM and Bové J-M 2019. Molecular characterization and detection of 16SrIII group phytoplasma associated with "huanglongbing" symptoms. *Phytopathology*, 109(3): 366-374.